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# Time course of cellular and molecular regulation in the immune system in altered gravity: Progressive damage or adaptation ?

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ABSTRACT

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## 1. Introduction

Gravity has been a constant factor throughout the evolution of life on Earth, and played an important role for the architecture and morphology of all biological systems. It can therefore be assumed that abrupt changes of the gravitational force have an impact on the function of living organisms. It is of great interest, if and how cellular and molecular functions adapt to gravitational changes or if they strictly depend on Earth's gravity. Many studies have been performed analyzing the effects of altered gravity on life from unicellular organisms to humans. In humans the current level of knowledge is that altered gravity, especially microgravity, leads to numerous deconditioning symptoms like bone demineralization, muscle atrophy, reduced performance of the cardiovascular system, altered neurovestibular perception as well as strong impairment of the immune system [1–4]. The dysregulation of the immune system under spaceflight conditions is described by vast number of reports [5–13]. The first observations were made already in the 1960s and 1970s where half of the Apollo astronauts developed bacterial or viral infections during spaceflight or shortly after returning to Earth [14]. Viral infections include also reactivation of latent viruses like the varicella zoster virus, cytomegalovirus and Epstein-Barr virus [15–19]. Furthermore, investigations of astronauts during and after their ISS (International Space Station) missions showed allergic hypersensitivity symptoms especially for the skin, indicating a loss of regulatory immune system function under spaceflight conditions [20,21]. At the cellular and molecular level post-flight dysregulation of the function of the human immune system has been reported frequently. Findings after landing include differences in immune cell subpopulations like e.g. in the distribution of peripheral blood leucocytes [22,23,7]. Furthermore, the reactivity of certain cell subsets is affected. Analyses of blood samples of nine astronauts after returning from the Skylab space station showed a reduced activation potential of lymphocytes upon mitogenic stimulation in comparison to the pre-flight data [24].

However, comparing the results of the numerous different missions, variable results were obtained for the different immune cell subsets [7]. Nevertheless, the collected data point out that cell populations are very sensitive to exposure to spaceflight conditions including launch and landing [7]. Monocytes, representing cells of the innate immune system, show a reduction in motility and a rearrangement of the cytoskeleton during spaceflight compared to 1 g in flight and ground controls [25]. T cells, representing the adaptive part of the immune system, are altered in their function. In Space Shuttle crewmembers, early T cell activation was elevated while in ISS crewmembers it was significant reduced after landing. In both cases, the ratio of secreted IFN-γ:IL-10 was decreased, indicating a Th2 shift in the astronauts' immune system [26]. The knowledge about the humoral immunity is sparse. A few studies described that immunoglobulin plasma levels are unaltered after short-term spaceflights [27–29]. Contradictory results are reported for long duration space missions. While one study described increased serum levels of IgA and IgG [30], another analysis showed that total serum levels of IgA, IgM and IgG were not significantly changed during long-term missions [29]. However, research in the amphibian *Pleurodeles waltl* showed no changes in the IgM heavy chain transcription but a threefold increase in the level of IgY heavy chain transcription, an analog to human IgG [31,32].

The dysfunction of lymphocytes, resulting in immune deficiencies, is thought to represent an enormous risk for long duration spaceflights [7]. Alterations in the immune response could cause a dysbalanced response to infections or cancer or lead to hypersensitivity reactions with severe clinical manifestations [33,34,6]. Hence, the underlying mechanisms of these immune dysfunctions need to be elucidated. Additionally to microgravity, the high psychological stress, as well as the high levels of radiation experienced in this extreme environment, represent environmental stressors. Nevertheless, based on numerous publications, there is strong evidence that microgravity is a factor that could be considered as major reason for an affected immune cell function during spaceflight [35–37]. During the last 40 years more and more studies came up investigating the influence of altered gravity on immune cells isolated from animal or human organisms. It could be shown that inter alia molecular mechanisms and signal transduction cascades are directly affected by microgravity or hypergravity. Hence, isolated lymphocytes are an ideal model to study direct primary effects of altered gravity at the cellular level without disturbing or interfering secondary and systemic influences of the entire organism. A combined approach of experiments performed on different microgravity platforms complemented with studies using ground based facilities [38] are important for the investigation of cellular and molecular processes influenced by altered gravity. Ground based facilities like clinostat, rotating wall vessel and random positioning machine, represent valuable tools since they often provide microgravity induced comparable results and therefore offer the possibility for relatively cost effective and fast investigations. Despite the enormous number of studies, we do not yet have a complete overview about the functions of the immune system in altered gravity including adaptation processes and reaching of new steady state levels [39].

This review provides an overview about the current status of knowledge of the effects of spaceflight on cells of the immune system with regard to adaptation in cell culture systems, animal models and human studies. We aimed to compile existing studies regarding the measured time points for potential adaptation effects.

## 2. Cellular studies

Many studies have been published where immune cells have been cultivated under spaceflight conditions with or without activation. The great advantage of cell culture studies is that direct and primary effects of altered gravity can be investigated on the cellular level. T cells, representing the adaptive immune system have been analyzed since long and are most likely the best investigated immune cells in cell culture.

Experiments that were performed under real as well as under simulated microgravity conditions showed that non-activated and activated T lymphocytes react sensitive to gravity with respect to cell cycle regulation [40], epigenetic [41] and chromatin regulation [42], differential gene expression [40,43] and micro RNA expression profile [44], cell motility [45,46], and regulation of apoptosis [47–49]. Furthermore, expression of cytokines such as interleukin- (IL-) 2, and interferon-gamma (IFN-γ) were changed in microgravity [50].

The effect of microgravity on the cell proliferation, maturation and function was analyzed *in vitro* by many groups. However, often only a single time point was measured and a time course monitoring the progress of the changes is missing – often due to technical and operational constraints. Table 1 summarizes the influence of microgravity and simulated microgravity on T cell proliferation after activation, a requirement for a functional immune response. The results of the different groups are listed according to the length of exposure to microgravity. *In vitro* activation of T cells under real microgravity was investigated only scarcely and is indeed described only in a few reports [43,51–53,38,54]. One describes the experiments from Cogoli and colleagues, which were performed during the Spacelab 1 mission in 1983 [51]. The reactivity

**Table 1**
T cell proliferation after stimulation under real and simulated microgravity conditions.

| T cell proliferation | Real microgravity | Simulated microgravity |
|---|---|---|
| ConA CD3/CD28 (activated) | ↓ 1.5 h [43] | ↓ 0.5 h [110] |
| | | ↓ 3, 24, 48 h [111] |
| | ↓ 71 h [51], [52], [53] | ↓ 24, 48, 72 h [112] |
| | ↓72 h [38] | ↓ 24, 48 h [113] |
| | ↓72 h [54] | ↓ 24, 48, 72 h [114] |
| | | ↓72 h [38] |
| PMA (activated) | – | ↓ 48 h [115] |
| CD3/IL-2 (activated) | – | ↓ 24 h [116] |

(↑) increase, (↓) decrease, (↔) no changes, (–) not analyzed, underlined arrows correspond to simulated microgravity experiments.

of the analyzed T lymphocytes upon stimulation with Concavalin A (ConA) was nearly completely abrogated after 71 h of microgravity [51,52] and after 72 h respectively [54,38]. Further experiments showed that the proliferation capacity of T cells in real microgravity is reduced already after 1.5 h [43]. A larger number of experiments have been performed under simulated microgravity with exposition times between 0.5 h up to 72 h (Table 1). For all analyzed time points the investigated T cells showed a clear reduction in their proliferation activity. This leads to the conclusion that T cells, at least within a time frame of 72 h, do not adapt to microgravity and are not able to restore the malfunction in cell proliferation. Interestingly, when ConA CD3/CD28 activated and exposed to hypergravity for 21 days, T cells reacted in a reversed mode with an increased proliferation rate [55].

Besides proliferation, T cell signaling has also been intensively investigated and it could be shown that microgravity influences the membrane proximal T cell receptor signaling (Table 2). Investigation of CD3, IL-2R, and LAT expression showed a reduced protein expression in activated and non-activated T lymphocytes during the first 6 min under real as well as under simulated microgravity [56,57]. Further studies concerning CD3 protein expression under simulated microgravity indicated that this decreased expression is balanced after approximately 30 min [57] (Table 2). For IL-2R the protein expression returned to normal values in activated T cells after 71 h exposure to real microgravity. So far experiments in simulated microgravity have been only performed up to 12 h [58] and results confirming an adaptation after 71 h incubation are still missing. For LAT and MAPK signaling results are rudimentary, and it cannot be concluded if there are adaptation processes. Furthermore, the influence of hypergravity on the membrane proximal T cell receptor signaling is mostly unknown. Only two reports exist describing a reduced protein expression in activated and non-activated T lymphocytes after 20 s and 43 s [56,57] (Table 2).

Cytokine release has also been intensely investigated in T cells under real and simulated microgravity. Cytokines function as signaling molecules and are important for inter-cellular communication and activation of immune cells and immune cell subsets. They are also important for the regulation of the extent of an immune response and therefore possess a rather short half-life and function at low concentrations. Table 3 shows the cytokine release of T lymphocytes under real and simulated microgravity at different time intervals. While IFN-γ, and TNF-α release was increased under real microgravity for all measured time points, studies under simulated microgravity showed the reverse effect and cytokine down regulation was observed in the experiments lasting between 24 h and 144 h. For IL-2 the largest number of data points were published. In real microgravity, down regulation of cytokine release (46 h) was followed by an increased secretion after 71 h and 72 h in microgravity, indicating either a compensatory effect or homeostasis at a new "steady state" level. IL-2 decrease but not increase after 71 h could be confirmed in simulated microgravity experiments (Table 3). Analyses for IL-1, IL-1b and IL-6 showed increased release values in simulated microgravity when T cells were stimulated via ConA CD3/CD28. However, under real microgravity, contradictory results were obtained after 24 h exposure when cells were stimulated with PMA or LPS. This result indicates that microgravity may affect the cellular target of phorbol ester [59]. Regarding PMA, this could be an indication that PMA may not work under microgravity conditions. It is also conceivable that activation of the PMA receptor, protein kinase C, was inhibited, either because PMA did not bind or because protein kinase C activation was blocked in microgravity. More studies about the activation of T cells under PMA are needed but still, there is an indication that intracellular signaling pathways are sensitive to gravity [59]. Licato et al. studied the influence of IL-2 activation and observed a down regulation of IFN-γ, TNF-α, and IL-1b after 48 h and 144 h. As the induction by IL-2 of secondary cytokines like IFN-γ, IL-1b, and TNF-α is an important aspect of IL-2 activation of immune functions, this abrogation suggests that IL-2 signaling pathways leading to various IL-2-mediated effects are differentially regulated under the tested conditions. A possible explanation could be the inhibition of CD25 (IL-2R alpha chain) upregulation in IL-2-stimulated cultures [60].

**Table 2**
Changes in protein expression after different time intervals in activated and non-activated T cells.

| T cells exposed to microgravity and simulated microgravity | CD3 | IL-R2 | MAPK | LAT |
|---|---|---|---|---|
| non activated | ↓ 20 s [57] | ↓ 20 s [57] | ↓ 6 min [56] | ↓ 20 s [57] |
| | ↓ 6 min [56] | ↓ 6 min [56] | | |
| ConA CD3/CD28 (activated) | ↓ 5 min, 15 min [57] | ↓ 6 min [56] ↓ 30 min | ↑ 20 s (MEK phosphorylation) | – |
| | ↓ 6 min [56] | 60 min [57] | [117] | |
| | ↔ 30 min, 60 min [57] | | ↑ 5 min [57] | |
| PMA (activated) | – | – | ↑ 20 s (MEK phosphorylation) [117] | – |
| | | | ↑ 5 min [57] | |
| 1–12 h microgravity | | | | |
| ConA CD3/CD28 (activated) | – | ↓4, 6, 8, 10, 12 h [58] | – | – |
| >12 h microgravity | | | | |
| ConA CD3/CD28 (activated) | – | ↔71 h [118] | – | – |
| T cells exposed to hypergravity | | | | |
| non activated | ↓ 20 s [57] | ↓ 20 s [57] | – | ↓ 20 s [57] |
| ConA CD3/CD28 (activated) | ↓ (5-7 g, 43 s) [56] | ↓ (5-7 g, 43 s) [56] | ↓ (5-7 g, 43 s) [56] | ↓ (5-7 g, 43 s) [56] |

↑ increase, ↓ decrease, ↔ no changes, (–) not analyzed, underlined arrows correspond to simulated microgravity experiments.

**Table 3**
Changes in cytokine release after different time intervals in activated and non-activated T cells.

| Microgravity and simulated microgravity | IFN-γ | IL-2 | TNF-α | IL-6 | IL-1b | IL-1 |
|---|---|---|---|---|---|---|
| ConA CD3/CD28 (activated) | ↑↑ 71 h [118]<br>↑↑ 72 h [54]<br>↓24, 48, 72 h [112] | ↓2, 4, 6, 8, 10, 12 h [58]<br>↓46 h [58]<br>↑↑ **71** h [118]<br>↑ 72 h [54]<br>↓24, 48, 72 h [112] | – | ↑↑24, 48, 72 h [112] | ↑↑24, 48, 72 h [112] | ↕ 4, 6, 8, 10, 12 h [58] |
| PMA (activated) | – | ↓ 24 h [59]<br>↓ 48 h [115] | – | – | – | ↓24 h [59] |
| LPS (activated) | ↑ 24 h [119] | – | ↑ 24 h [119] | – | – | ↑ 24 h [119] |
| IL-2 (activated) | ↓ 48, 144 h [60] | – | ↓48, 144 h [60] | – | ↓48, 144 h [60] | – |

↑ increase, ↓ decrease, ↔ no changes, (–) not analyzed, underlined arrows correspond to simulated microgravity experiments.

Taken together, we see that there are numerous gaps in the investigation of the influence of real and simulated microgravity on the cytokine release of activated T cells with respect to the time course from short-term to long-term microgravity. In the case of ConA CD3/CD28 T cell stimulation and IL-2 release, where the most data are present, indications for adaptation processes are visible after approximately 3 days. Research in this field should be intensified, especially using the cost effective ground based simulated microgravity facilities to investigate in more detail if and when adaptation processes appear.

Studies published about other immune cell types include natural killer (NK) cells, which remained unaltered in their function in cell culture [61] as well as monocytes and macrophages. The cell line J-111 was functionally impaired during spaceflight and a distinct reduction in the cellular motility could be observed compared to 1 g in-flight and ground controls [25]. In macrophages a rapid and reversible response to altered gravity was observed [62,63]. Macrophages belong to the monocyte-macrophage-system (MMS) of the body's innate immune system and represent the first line of defense against microbial infections. The effectiveness of reactive oxygen species (ROS) production upon stimulation was investigated in a combined approach using 2D clinorotation and real microgravity on parabolic flights. The macrophageal oxidative burst, which is decreased upon microgravity and increased by hypergravity [62], is one of the key elements of the innate immune response and cellular signaling [64]. However, long-term functional analyses in real microgravity are still missing for this type of immune cells.

Cell culture experiments represent most likely the best and most cost effective option to generate a reliable *in vitro* basis of data for analyses of cells of the immune system exposed to real and simulated microgravity. Especially ground-based facilities like clinostats represent an excellent way to perform continuous experiment timelines from minutes to hours and days to investigate the effect of altered gravity on cells of the immune system and to discover adaptation effects and new levels of homeostasis. Surprisingly, *in vitro* studies are rare and were performed mainly as short-term studies with data points until 3 days, indicating that the experimental potential of *in vitro* studies is not exhausted by far, in particular with regard to ground-based clinostat studies.

## 3. Animal models

Animals, especially mice and rats represent widely used model organisms to elucidate biological phenomena and mechanisms and to better understand disease processes. Research in rats and mice have been deployed in rather short-term experiments of up to approximately two weeks microgravity because of lack of technical facilities for long duration flights. With respect to the proliferation rate of T lymphocytes, no significant changes after 8 days in rats [65] and 15 days in mice [66] could be observed. In experiments that have been performed to determine the effects on the cytokine production, cells were isolated from flown animals shortly after landing and stimulated with mitogens to induce cytokine secretion. The results in Table 4 display that the production of different cytokines is not uniformly affected by spaceflight. The immune-regulatory and antiviral cytokine IFN-γ is initially increased after one to two days in microgravity. Thereafter, from day 12 onwards the cytokine release is decreased, indicating an adaptation to the new environment. In contrast, after 21 days in hypergravity, the IFN-γ secretion is significantly increased (see Table 4). TNF-α release was also elevated in mice after one and two days in microgravity. After 12 days in microgravity, TNF-α secretion returned to a normal level when compared with ground control animals and IL-2 was down regulated. However, the careful interpretation of data is required, because the early data points were recorded for mice and the later data points for rats. For IL-1, IL-4, IL-5, IL-6, IL-10, IL-17A, no prediction of an adaptation or a persisting dysbalance is possible because of lacking data points.

## 4. Astronaut studies

Several reports describe severe effects of spaceflight on the astronauts' immune system comparing between pre- and

**Table 4**
Changes in cytokine release after different time intervals in activated T cells of animal models.

| Microgravity | IFN-γ | IL-4 | IL-5 | IL-10 | IL-2 | IL-17A | TNF-α | IL-6 | IL-1 |
|---|---|---|---|---|---|---|---|---|---|
| ConA CD3/CD28 (activated) | ●↑ 1d,2d [119]<br>●↓**12d** [120]<br>●↓ 15d [121] | ●↓12d [120] | ● ↔ 12d [120] | – | ▲ ↔ 8d [66]<br>▲ ↔ 10d [28]<br>●↓ **12d** [120]<br>●↓ 15d [121] | – | ●↑ 1d,2d [119]<br>● ↔ **12d** [120] | – | ●↑ 1d,2d [119] |
| PMA (activated) | – | – | – | – | ▲↓10d [28] | – | – | – | – |
| LPS (activated) | – | – | – | ●↓13d [122] | – | – | ● ↔ 13d [122] | ●↓13d [122] | – |
| Hypergravity | ● ↑↑ 21d [55] | ● ↔ 21d [55] | ● ↔ 21d [55] | ● ↔ 21d [55] | ●↓↓21d [55] | – | – | – | – |

●Mouse ▲Rat, ↑ increased ↑↑ strongly increased, ↓ decreased ↓↓ strongly decreased ↔ no change.

post-flight data, directly before launch and after landing, where dysfunctions regarding cell proliferation as well as for cell functions like cytokine release have been observed [22,26,8,30]. However, due to the diverse stress factors that affect astronauts directly before and during launch and landing, as well as during the spaceflight [17,19,36], other factors in addition to the gravitational changes might have a significant influence on the organism and might superimpose the direct effects of microgravity. Recently, new investigations, including baseline data recordings before and after spaceflight as well as in flight sampling at different time points, provided new insights into the function of the human immune system during spaceflight.

The following tables display the results of three different studies concerning the changes in cytokine levels. Table 5 summarize the cytokine concentration in the blood plasma of astronauts before, during and after a short duration spaceflight of 10 to 15 days [9]. Samples were taken 180 days before launch (L-180), at L-10, during the flight (FLT; i.e. the day before landing) and directly after landing (R + 0) and 14 days post-flight. Data are displayed as differences in cytokine levels. Two consecutive data points were compared with each other and the cytokine up- or down-regulation was displayed with arrows. For all measured cytokines, an increase after 10 to 15 days spaceflight could be detected including IL-1β and IFN-α, which were considerably increased upon exposure to microgravity. Blood plasma levels of all cytokines decreased immediately after landing, except for IL-6, which decreased only 14 days post-flight. 14 days after landing, the last measured time point, the plasma levels of IL-1β, IL-4, IL-6, IL-10, IL-12 and IL-17 returned to their pre-flight level. TNF-α, IFN-α and IFN-γ showed higher plasma concentrations 14 days after landing compared to L-180. The concentration of IL-6 was significantly increased directly after landing while the plasma levels of all other measured cytokines were significantly increased during spaceflight. Measurements of in-flight cytokine profiles (IFN-α, IFN-γ, IL-1β, IL-4, IL-10, IL-12 and TNF-α) could clearly separate in-flight dysregulation from stress induced alterations immediately after landing (Table 5). Table 6 summarizes the mean intracellular and secreted cytokine concentrations in stimulation assays: To monitor intracellular cytokines, PMA/ionomycin was used as stimulant for 4 h. For the detection of secreted cytokines, cells were stimulated for 48 h with either *anti*-CD3/CD28 (T cell specific stimulation), LPS or PMA/ionomycin. IFN-γ, IL-10 and IL-17A were significantly reduced in-flight while IL-2 levels were significantly elevated. TNF-α after *anti*-CD3/CD28 or LPS stimulation was significantly elevated while TNF-α after PMA/ionomycin stimulation was decreased in-flight. After PMA/ionomycin stimulation, IL-4, IL-5 and IL-6 were significantly decreased in-flight. Following LPS stimulation, IL-8, IL-1β, and IL-6 were increased during flight, whereas IL-12 and IL-4 production was unaltered after *anti*-CD3/CD28 stimulation. Most of the cytokines that were dysregulated during flight remained also dysregulated after landing, whereas for IFN-γ and IL-6 a tendency towards baseline was observed.

Further investigations with 28 ISS astronaut crewmembers included additional measurement points before and during flight. Cytokine blood plasma levels were measured at L-180, L-45, L-10, flight day 15 (FLTd15), FLTd30, FLTd60, FLTd120, FLTd180, R + 0, and R + 30 [67]. IL-1α, IL-6, IFN-γ, IL-2, IL-17, IL-4, IL-5, IL-10, and GM-CSF concentrations were below or only marginally above the sensitivity threshold, therefore no evaluation of cytokine changes were possible. Fig. 1 summarizes the mean plasma

**Table 5**
Differences in blood plasma cytokine concentrations in astronauts during spaceflight (data adapted from [9]).

| Cytokines | L-10 vs. L-180 | FLT vs. L-10 | R + 0 vs. FLT | R + 14 vs. R + 0 |
|---|---|---|---|---|
| IL-1β | ↑ | ↑↑↑ | ↓↓ | ↓ |
| TNF-α | ↑ | ↑ | ↓ | ↑ |
| IL-6 | ↑ | ↑ | ↑ | ↓ |
| IL-12 | ↑ | ↑ | ↓ | ↑ |
| IFN-α | ↑ | ↑↑ | ↓ | ↑ |
| IFN-γ | ↑ | ↑↑ | ↓ | ↑ |
| IL-4 | ↑ | ↑ | ↓ | ↔ |
| IL-10 | ↑ | ↑ | ↓ | ↓ |
| IL-17 | ↑ | ↑ | ↓ | ↓ |

↑ increase ↓ decrease ↔ no change, ↑ weak ↑↑ moderate ↑↑↑ strong.

**Table 6**
Intracellular and secreted cytokine concentrations in stimulation assays, blood sample from astronauts after different time points in altered gravity conditions (data adapted from [9]).

| Cytokines | L-10 vs. L-180 | FLT vs L-10 | R + 0 vs. FLT | R + 14 vs. R + 0 |
|---|---|---|---|---|
| CD4/IL-2 (PMA/ionomycin) | ↓ | ↔ | ↓ | ↑ |
| CD8/IFN-γ (PMA/ionomycin) | ↑ | ↓ | ↓ | ↑ |
| IFN-γ (*anti*-CD3/CD28) | ↑ | ↓↓↓ | ↑ | ↑↑ |
| IFN-γ (PMA/ionomycin) | ↓ | ↓ | ↓ | ↑↑ |
| TNF-α (*anti*-CD3/CD28) | ↑ | ↑↑ | ↓↓↓ | ↑ |
| TNF-α (PMA/ionomycin) | ↓ | ↓↓ | ↑ | ↑↑↑ |
| TNF-α (LPS) | ↑ | ↑↑↑ | ↓↓ | ↓ |
| IL-10 (*anti*-CD3/CD28) | ↓ | ↓↓↓ | ↑↑↑ | ↓ |
| IL-10 (PMA/ionomycin) | ↓ | ↓↓↓ | ↑↑↑ | ↑↑↑ |
| IL-10 (LPS) | ↔ | ↓↓↓ | ↑↑ | ↓↓ |
| IL-4 (*anti*-CD3/CD28) | ↔ | ↔ | ↔ | ↔ |
| IL-4 (PMA/ionomycin) | ↔ | ↓↓↓ | ↔ | ↑↑↑ |
| IL-5 (*anti*-CD3/CD28) | ↑ | ↓ | ↔ | ↑↑↑ |
| IL-5 (PMA/ionomycin) | ↓ | ↓↓ | ↔ | ↑↑↑ |
| IL-2 (*anti*-CD3/CD28) | ↑↑ | ↑↑ | ↓↓↓ | ↑↑↑ |
| IL-2 (PMA/ionomycin) | ↓ | ↑ | ↓ | ↑ |
| IL-17A (*anti*-CD3/CD28) | ↓↓↓ | ↓↓↓ | ↑↑↑ | ↑↑ |
| IL-17A (PMA/ionomycin) | ↑↑ | ↓↓↓ | ↑↑↑ | ↑↑↑ |
| IL-6 (*anti*-CD3/CD28) | ↑ | ↑ | ↓ | ↓ |
| IL-6 (PMA/ionomycin) | ↑↑ | ↓↓↓ | ↑↑ | ↑↑ |
| IL-6 (LPS) | ↓ | ↑ | ↔ | ↓ |
| IL-12 (LPS) | ↔ | ↔ | ↔ | ↔ |
| IL-1β (LPS) | ↓ | ↑↑↑ | ↓ | ↔ |
| IL-8 (LPS) | ↑ | ↑ | ↑ | ↓ |

↑ increase ↓ decrease ↔ no change ↑ weak ↑↑ moderate ↑↑↑ strong.

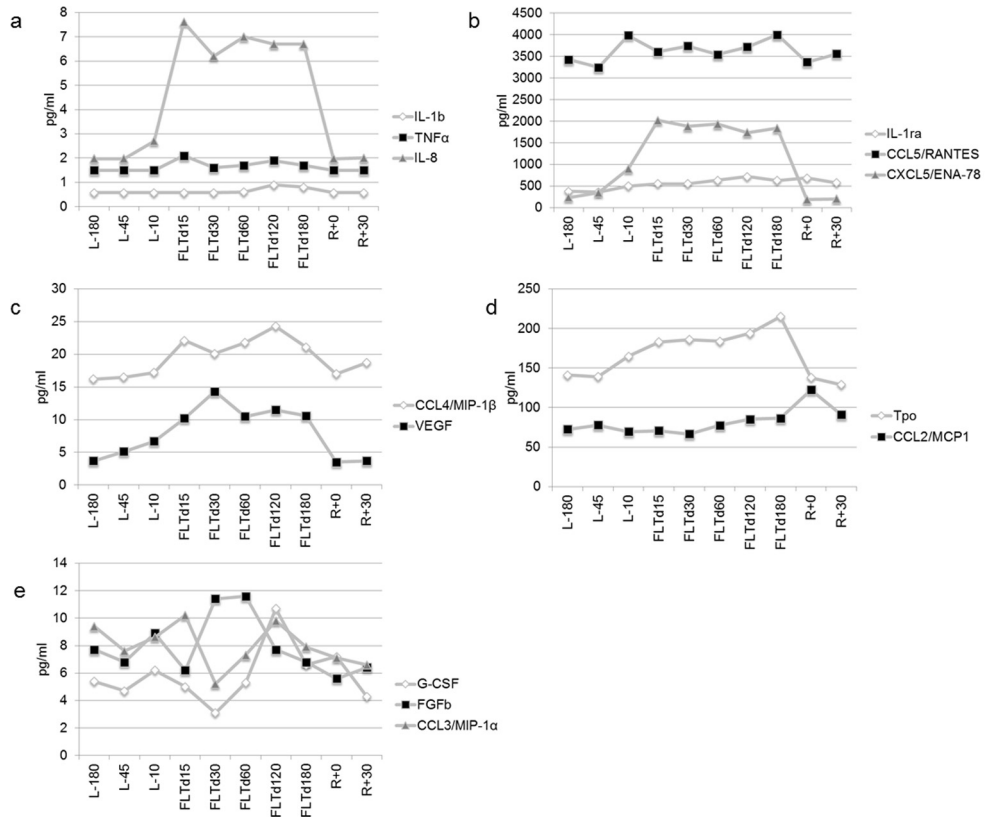


**Fig. 1.** Plasma cytokine concentrations during long duration spaceflight. Data are adopted from [69] and the published tabular values were plotted in diagrams (a–e). The mean plasma cytokine concentrations were expressed as mean concentrations (pg/ml) of the respective cytokine in 28 astronauts. Values that were constantly below or only marginally above the indicated sensitivity threshold were not included in the diagrams. When cytokine concentrations were below the detection limit, the sensitivity threshold was displayed (valid for IL-1b: L-180, L-45, L-10, FLTd15, FLTd30; TNFα: L-180, L-45, L-10; IL-8: L-180, L-45). a) IL-1b showed only minor in-flight alterations, while TNFα and IL-8 were clearly increased from FLTd15 on. b) For IL-1ra no major changes could be observed except a slight trend of increased expression, which was visible from L-10 onwards. For CCL5/RANTES the measured data points were rather fluctuating and no obvious trend was detectable throughout the measurement. Blood plasma concentrations for CXCL5/ENA-78 increased clearly already at FLTd15 and stayed increased until return to Earth (R + 0). c) VEGF and CCL4/MIP-1β levels increased both in-flight from FLTd15 onwards. d) Tpo concentrations in blood plasma showed slowly but steadily increasing values, while CCL2/MCP1 was rather stable in-flight but increased at R + 0. e) G-CSF, FGFb and CCL3/MIP-1α showed a rather fluctuating data situation as they respond with an over- and undershooting reaction respectively before they showed the tendency to return to the baseline values during flight.

cytokine concentrations (pg/ml) in astronauts before, during, and after spaceflight from Table 2 from [69]. The published tabular values for the mean plasma cytokine concentrations were plotted and displayed in diagrams. Values within the same order of magnitude were combined in a common graph (Fig. 1a–e). Fig. 1 shows that cytokines were mostly increased after 15 days of spaceflight (TNF-α, IL-8, IL-1ra, CCL5/RANTES, CXCL5/ENA-78, CCL4/MIP-1β, VEGF, Tpo). However, only IL-8, Tpo and CXCL5/ENA-78 were significantly elevated during nearly all time points during spaceflight, whereas IL-1ra, VEGF and CCL4/MIP-1β demonstrated at least one significant increase during flight. TNF-α was not significantly altered. IL-8 and CXCL5/ENA-78 remained elevated until flight day 180, indicating a new steady state level, whereas, Tpo secretion increased constantly until FLTd180 without reaching a steady state in the measured time interval. In contrast, G-CSF, Granulocyte-Colony Stimulating Factor, was first decreased until FLTd30, when compared to the baseline level (L-180), then increased until FLTd120, and thereafter reduced at FLTd180 and returned to baseline at R + 30. FGFb, basic fibroblast growth factor, concentration decreased at FLTd15, followed by a twofold increase until FLTd60, before it returned to baseline levels between FLTd120 and FLTd180. However, alterations of FGFb and G-CSF were not significant in statistical tests. CCL3/MIP-1α, macrophage inflammatory protein 1-alpha, was first slightly increased compared to the baseline level (L-180), then reduced at FLTd30 and returned to baseline level at FLTd180, however also with non-significant values. Overall, single data points of individual astronauts demonstrated a strong variability in cytokine concentrations during spaceflight conditions.

Crucian and colleagues published another very valuable study investigating the effect of long duration spaceflight on the cytokine release of mitogen stimulated PBMCs [10]. Blood samples were collected at L-180, L-45, FLTd14 (early), FLT 2 to 4 months (mid-mission), FLT 6 months (late), R + 0, and R + 30 (Table 7). PBMCs were stimulated in culture under the presence of either *anti*-CD3/CD8 to activate T cells via their T cell receptor, PMA/ionomycin representing a broader pharmacological stimulus, and lipopolysaccharide (LPS) for monocyte activation. Table 7 summarizes the results of the cytokine release from stimulated PBMCs. Concentrations of IFN-γ, IL-4, IL-5, IL-10, IL-17A, TNF-α, and IL-6 were all significantly decreased at all three in-flight time points [10]. Following stimulation with LPS, no significant decreases were observed during flight, but the concentration of IL-8 was actually increased at all three in-flight time points [10]. It could be hypothesized that short-term microgravity tends to inhibit human T cell function during the first days and weeks. This is also in line with data obtained from cell culture and animal models. Then, after two until four months under microgravity conditions, an adaptation process seemed to start in T cells, where the lowest point in cytokine release was detected for most of the measured

**Table 7**
Cytokine release from PBMCs isolated from astronauts after mitogen stimulation (data adopted from [10]). The published data were resumed by comparing two consecutive data points [10] and the increase or decrease of cytokine secretion was displayed with up or downward directed arrows. Early = FLT 14 days, Mid = FLT 2–4 months, Late = FLT 6 months.

| Cytokines | L-45 vs. L-180 | Early vs. L-45 | Mid vs. Early | Late vs. Mid | R + 0 vs. Late | R + 30 vs. R + 0 |
|---|---|---|---|---|---|---|
| IFN-γ (*anti* CD3/CD28) | ↑↑ | ↓↓↓ | ↑↑ | ↑↑ | ↑↑ | ↑↑ |
| IFN-γ (PMA/ionomycin) | ↓ | ↓↓↓ | ↑↑ | ↑↑ | ↑↑ | ↑↑ |
| IL-4 (*anti* CD3/CD28) | ↑ | ↓↓ | ↓↓ | ↑ | ↑↑ | ↑↑ |
| IL-4 (PMA/ionomycin) | ↑ | ↓↓↓ | ↑ | ↑ | ↑↑ | ↑↑↑ |
| IL-5 (*anti* CD3/CD28) | ↓ | ↓↓ | ↑ | ↑ | ↑↑ | ↑ |
| IL-5 (PMA/ionomycin) | ↓ | ↓↓↓ | ↑ | ↑↑ | ↑↑ | ↑↑ |
| IL-10 (*anti* CD3/CD28) | ↑ | ↓↓↓ | ↑ | ↑ | ↑↑↑ | ↑↑ |
| IL-10 (PMA/ionomycin) | ↓↓ | ↓↓↓ | ↑ | ↑ | ↑↑ | ↑↑ |
| IL-2 (*anti* CD3/CD28) | ↑↑ | ↓↓ | ↑↑ | ↑↑ | ↓↓↓ | ↑↑ |
| IL-2 (PMA/ionomycin) | ↓ | ↓↓↓ | ↑↑ | ↑↑ | ↑↑ | ↑ |
| IL-17a (*anti* CD3/CD28) | ↓↓ | ↓↓↓ | ↑ | ↑ | ↑↑ | ↑↑ |
| IL-17a (PMA/ionomycin) | ↓↓ | ↓↓↓ | ↑ | ↑ | ↑ | ↑↑↑ |
| TNF-α (*anti* CD3/CD28) | ↑ | ↓↓ | ↑↑ | ↓ | ↓ | ↑↑↑ |
| TNF-α (PMA/ionomycin) | ↓↓ | ↓↓↓ | ↑ | ↑ | ↑↑ | ↑↑ |
| IL-6 (*anti* CD3/CD28) | ↑↑ | ↓↓ | ↓↓ | ↑ | ↑↑ | ↔ |
| IL-6 (PMA/ionomycin) | ↓ | ↓↓↓ | ↑ | ↑ | ↑↑ | ↑ |
| TNF-α (LPS) | ↔ | ↓ | ↓↓ | ↓↓ | ↑ | ↑↑ |
| IL-6 (LPS) | ↓ | ↓↓ | ↑↑ | ↓↓ | ↑↑ | ↑↑ |
| IL-1b (LPS) | ↓↓ | ↓↓↓ | ↑↑ | ↓↓ | ↑↑ | ↑ |
| IL-8 (LPS) | ↑ | ↑↑ | ↑ | ↓↓ | ↓↓ | ↑↑ |
| IL-10 (LPS) | ↑↑ | ↓↓↓ | ↓ | ↑↑ | ↑↑ | ↑↑ |

↑ increase ↓ decrease ↔ no change, ↑ weak ↑↑ moderate ↑↑↑ strong.

parameters. In case of IL-4 (*anti*-CD3/CD28), and IL-6 (*anti*-CD3/CD28), a possible turning point is visible only after 6 months in microgravity (late). The measurements for IL-2 (*anti*- CD3/CD28) and TNF-α (*anti*-CD3/CD28) secretion showed rather fluctuating results, making an evaluation of potential adaptation processes difficult. The stimulation of monocytes by LPS also showed rather heterogenic results: The concentrations for IL-1β and IL-6 are fluctuating, whereas TNF-α secretion was constantly decreased until R + 0. For IL-8 and IL-10, an inversion of the cytokine release and therefore a possible trend for an adaptation process was visible only in the late phase of microgravity exposure after 6 months.

## 5. Countermeasures

Long duration spaceflights to another planet as planned by NASA and other space agencies represent a risk for the health status of the astronauts. The immune system is challenged during space travel by various factors such as isolation or confinement [68], psychological stress, altered nutrition, altered microbial virulence [69,70,71,72], altered microbiomes [73], increased biofilm formation [74], circadian misalignment, radiation, and microgravity [75]. The effects are manifold and affect the innate as well as the adaptive immune system [76,26,7,15].

Nowadays countermeasures applied to reduce the risk for astronauts to develop infectious diseases are resumed in the so-called Health Stabilization Program (HSP). Astronauts are isolated preflight and subjected to quarantine 7 days before launch (L-7) where they have only limited contact to other individuals to reduce the infection risk. The HSP also includes surveillance of the astronauts' health status by blood and urine sample analyses of the crewmembers [75,77]. Additionally, the medical and dental history is analyzed as well as the vaccination status for influenza, tetanus, diphtheria, pertussis, mumps, measles, and rubella. Furthermore, the astronauts are screened for tuberculosis, *S. aureus* and HIV infections [77]. Not only the crewmembers are intensely screened but also the spacecraft and the space station, which could be contaminated by microorganisms [78]. Microbes have been identified in free-floating condensates aboard the Mir space station [79]. Therefore, aerosols produced by coughing or speaking represent a danger for transmission of viruses like influenza [80] and bacteria such as *S. aureus* [81] from one crewmember to another. Countermeasures are also applied considering the equipment of the space vehicle and the space station. The air is filtered by a high-efficiency particular air (HEPA) filter and the air humidity is controlled [77]. Water filters that are utilized and improved contamination resistant surfaces should be implemented [82]. Besides these hygienic and environmental controls, astronauts are obliged to do regular exercise, which could be also beneficial for the function of the immune system during space travel [83].

However, this is not sufficient to cope with the above-mentioned spaceflight effects that are likely to be associated with decreased resistance to viral and bacterial infections as well as an increase in allergic and autoimmune complications [84]. Further prophylaxis treatment was proposed regarding nutritional measures like the intake of substances, which have several positive effects such as modulation of the innate and adaptive immunity [85,86], benefits on the response to vaccination for influenza [87], polio [88] and cholera [89]. Additionally, it could be shown that some plant derived medicines and herbal products caused immune modulatory responses like e.g. Saikosaponin from *Bupletum falcatum* which increased the IL-2 production [90] and *Silybum marianum* derived from the milk thistle, which acts immunostimulatory [91]. Furthermore, application of nutritional supplements showed promising results: In particular, molecules with antioxidant properties are promising countermeasures against oxidative stress that is associated with spaceflight and radiation exposure [92–94]. An anti-oxidative and protective function against space radiation induced oxidative stress could be shown for N-acetyl cysteine, ascorbic acid, α lipoic acid, L-selenomethionine, coenzyme Q10 and vitamin E succinate [95,96]. Kennedy and colleagues could also show that administration of D-selenomethionine reduced the irradiation induced decrease of total antioxidants [97]. Tocopherol, has been shown to be effective in correcting altered IL-6 production and in decreasing synthesis of acute phase proteins [98], [99]. Vitamin D supplementation led to an enhanced immune function [100] and reduced the risk of herpes virus reactivation [101].

The effects of nutritional nucleotide supplementation on the immune function was investigated by Kulkarni and colleagues [102,103]: Mice were exposed to simulated microgravity effects

by hindlimb unloading, which resulted in significant suppression of immune function and decreased IL-2 and IFN-γ concentrations. These effects were restored when food was supplemented with RNA or uracil for a time span of 7 days. The administration of the supplemented chow could restore various immunological functions [103,104], such as cell proliferation and cytokine production [105,106,104], reduced corticosteroid levels [103], regulated T cell growth and – function [103,104], increased cell locomotion [103], decreased melanoma metastasis and improved wound healing [104]. This leads to the conclusion that in stress situations such as spaceflight, substitute food has highly regulating and immunoprotective effects and could therefore represent an important countermeasure for immune dysfunction in space [103].

Another interesting substance is active hexose correlated compound (AHCC), an alpha-glucan, produced by the mycelia of Basidiomycete mushrooms. This compound enhanced the resistance of hindlimb unloaded mice to *Klebsiella pneunomiae* infection [107] as well as the TH1 response in ConA activated splenocytes [108]. Administration of non-purified leukocytic and fibroblast interferon, restored the NK cell activity in mice after exposure to a stressor [109].

Successful results have also been achieved with the therapeutic use of biological response modifiers (BRM). The following approaches showed promising results in the treatment of cancer (CSF and IFN-γ, recombinant mutant TNF-S), infectious diseases (TNF against Hepatitis B) and autoimmune reactions (CSF against autoimmune neutropenia) [68].

Applications included:

- induction of cell maturation and differentiation (erythropoietin, thymus hormones, colony-stimulating factors CSFs)
- cytokines and recombinant mutants (interleukins IL-2 and IL-4), interferons, TNF-α
- natural and synthetic immunomodulators (LPS, Muramyldipeptide MDP, lectins)
- cells like natural killer (NK) cells, lymphokine-activated killer (LAK) cells, tumor-infiltrating lymphocytes (TILs), cytotoxic T lymphocytes (CTLs)
- cytostatic agents

A very effective approach of modern immunotherapy is also the application of artificial vaccines, monoclonal antibodies and immunoconjugates, recombinant cytokines, or immunomodulators, mostly applied in transplantation and cancer medicine [68].

The currently most common treatment in astronauts during an infection is the administration of antibiotics. However, bacteria in microgravity showed an enhanced resistance to antibiotics. Therefore the above-mentioned substances and methods have to be further evaluated and new combinations and strategies have to be analyzed to handle this problem. It will be important to find suitable compilations of substances and methods to mitigate the negative effects of spaceflight on the human body and to enable astronauts to perform long duration spaceflight as required for a mission to another planet.

## 6. Conclusion

Taken together these studies indicate an adaptation reaction of the immune system to the new microgravity environment, at least for the T cell system. Animal and human studies indicated adaptation processes starting after two weeks and continuing until 6 months or longer, which was reflected by cytokine concentrations in blood plasma or in stimulation assays. Adaptive reactions regarding IFN-γ, TNF-α and IL-2 concentrations were detected after 12 days of spaceflight in animal studies and after 2–4 months in human studies, whereas adaptive reactions regarding IL-4, IL-6, IL-8 and IL-10 were found after 6 months spaceflight. Interestingly, IL-8 and CXCL5/ENA-78 remained significantly elevated during the entire spaceflight, both cytokines are important regulators of neutrophil recruitment. Importantly, cellular studies were performed mainly as short-term studies, and only a few studies addressed alterations longer than 3 days. Long-term *in vitro* studies are completely missing. Therefore, more detailed and prolonged data analysis with narrow-meshed data points are necessary to identify and understand adaptation mechanisms of the immune system in altered gravity. The current knowledge is mainly based on single data point analysis in studies with a great variability in design, and lack of systematic reciprocal validation across the different model systems. Of course, this situation may be caused at least partially by the operational, technical and administrative constraints of spaceflight experiments.

Although many dysfunctions of the immune system have been described in astronauts during short duration spaceflight, the amount of medical incidences is limited and the immune problems are rather minor during short and long duration flights. So far no live threatening event has been reported during the last decades associated with a malfunction of the immune system. Observed symptoms include: allergic reactions, prolonged congestion, rhinitis, sneezing, cold sore, ear related pain, congestion and itchiness, pharyngitis, skin infection, skin rash and hypersensitivities, urinary tract infections and other infections (Table 8). This observation leads to the assumption that adaptive responses of cells and whole organisms could be expected under microgravity and altered gravity in general.

We summarized the current knowledge about adaptation processes of isolated immune cells, animal models and the human body to altered gravity conditions. Cross validation between *in vitro* and *in vivo* studies is often not possible or indicated conflicting results. For example, T cell proliferation was decreased *in vitro* and without indications of adaptions, confirmed in many studies, whereas no significant decrease in T cell proliferation was detected *in vivo* in rat and mice after 8 and 15 days of spaceflight, respectively. Blood cell counts in astronauts showed only minor changes in general lymphocyte and T cell counts [10]. Concerning different T cell subsets, a heterogeneous situation was observed. However, an overall down regulation trend was visible when compared to baseline values (L-180) [10]. T cell proliferation studies from astronauts that experienced long duration flights are mostly lacking. T cell signaling was mostly studied in cell culture systems in real and simulated microgravity in short time frames until 3 days. Up to now, we have no knowledge about molecular alterations of T cell signaling or adaptation responses in the signal transduction pathways when exposed to long-term microgravity. In most of the published studies, cytokine concentration in human blood plasma and in cell culture supernatants from T cells isolated from animal models or from astronauts were analyzed. Nevertheless, the results are fragmentary and further research is required

**Table 8**
In-flight clinical symptoms, ISS Program through 38 Expeditions; 46 ISS crew members (6-month missions); total flight duration 7508 days/20.57 flight years (adopted from [21]).

| Condition/symptoms | Total events |
|---|---|
| Allergic reaction | 2 |
| Prolonged congestion, rhinitis, sneezing | 20 |
| Herpes virus (cold sore) | 6 |
| Ear related: pain, congestion, itchiness | 6 |
| Pharyngitis (sore throat) | 1 |
| Skin infection | 6 |
| Skin rash/hypersensitivity | 23 |
| Urinary tract infection | 2 |
| Infections and others | 4 |
| Total | 70 |

to fill the existing gaps, in particular coordinated approaches and standardized protocols could support cross-validation of the pace and extent of adaption response in the human immune system, following *in vitro* and *in vivo* approaches. Such knowledge is of crucial importance for risk assessment, systematic and validated medical monitoring and potential countermeasures during exploration class missions.

Because gravity has been constant throughout the history of Earth and evolution of life [123], no pre-set adaptation program can be expected and the cellular response may therefore be less organized than other adaptation processes. Indeed, a plethora of *in vitro* studies, mostly done with T lymphocytes, demonstrated extensive alterations in almost every cellular and molecular aspect which were examined in more detail [40–57]. In contrast, long-term studies with animals and humans are completely lacking this dramatic picture of short-term cellular effects, which indicates a very efficient adaptation process, partially evidenced by new steady state of adaptive response in the human immune system after weeks until months [69]. Therefore, we assume that the human body and its cells are equipped with a robust and efficient adaptation potential when challenged with low gravitational environments.

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